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Assessment of Rhizobia Strains Isolates of Soils around Lake Victoria Basin for their Effectiveness in Nodulation and Symbiotic Efficiency on Soybeans and Bambara Groundnuts

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Abstract**—** *The symbiotic Biological nitrogen fixation (SBNF) is a sustainable and low-cost alternative to expensive and inaccessible inorganic fertilizers. However, SBNF is underutilized in soils of Lake Victoria basin due to insufficient information on local rhizobial strains diversity and their N-fixation efficiency. This study was carried out to assess the effectiveness of rhizobium strains isolates of Kisumu, Port Victoria, Kendu bay and Karungu soils within Lake Victoria basin in nodulation and symbiotic efficiency on soybeans and bambara groundnuts. Two bambara seeds of groundnut landraces; Kakamega Cream (KAKC) and Busia Brown (BUSB) used in this study were collected from farmers in Kakamega and Busia counties respectively. Screen house experiment was performed at Kenya Forestry Research (KEFRI) in plastic pots with four seeds of each cultivar which was later thinned to two plants. Randomized Complete Block Design (RCBD) was used. Experiments data were subjected to analysis of variance (ANOVA) using Genstat 16th Edition and significant means separated using Least Significant Difference at [$LSD_{5\%}$] and Dancun Multiple Range Test (DMRT). Result indicated highly significant ($p<0.05$) effect of isolate inoculation on number of nodules per plant. Soybean Variety SB19 formed effective nodules with rhizobia in the genera Bradyrhizobium, Rhizobium and Agrobacterium. On the other hand, 'Safari' was quite selective and formed very few nodules with isolates identified as Bradyrhizobium. However, both varieties SB19 and 'Safari' had better growth under glasshouse inoculation with Bradyrhizobium spp., rhizobia isolates although one Rhizobium isolate (SoyKis1) resulted in good nodulation of both varieties. Seed treatment of the two legumes with some isolates resulted in improved nodulation and better plant growth; in some instances, outperforming the commercial strain Bradyrhizobium japonicum USDA110. In conclusion, Isolates BAMKis12, BAMKis8, BAMKis4, BAMKbay8 and SoyKar2 were found to be potential elite strains and are recommended for more host range tests as viable inoculants sources.*

Keywords**—Rhizobia, Nodulation Effectiveness, Soybeans, Bambara Groundnuts.**

I. INTRODUCTION

Strategies to increase soil N-fertility as an alternative to expensive and inaccessible inorganic fertilizers lie in the use of symbiotic Biological nitrogen fixation (BNF). Symbiotic BNF is an important source of soil N. Legume crops have been shown to fix as much as 300 kg/ha/yr [1][2]. Globally, symbiotic N fixation has been estimated to amount to at least 70 million metric tonnes of nitrogen per year [3], with higher values of up to 90 million metric tonnes [4]. This is a proof that biological nitrogen fixation has significantly reduced the dependence of agriculture on N fertilizers [5]. The nitrogen in amino acids, purines, pyrimidines and other biomolecules ultimately comes from atmospheric nitrogen (N_2) and has to been converted into forms available for living organisms

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[6]. The biosynthetic process of conversion starts with reduction of N_2 into NH_3 (ammonia) in a process called nitrogen fixation by the rhizobia bacteria [7]. Majority of legumes have a distinctive symbiotic association with N-fixing rhizobia [8]. The symbiosis results in the plant roots being invaded by the bacteria to form organized plant root structures known as root nodules [9]. Effective use of this association requires knowledge of indigenous rhizobia that can effectively nodulate with legumes such as soybeans and bambara groundnuts cultivated in the region [10]. This information which is required for selection of competitive indigenous strains for use as bio-fertilizers in seed inoculation programmes is currently limited.

Commercial inoculants available in the market are made from exotic strains most of which may be less adapted to the local soils than indigenous strains. As a result, the potential of local strains to optimize BNF for improved soil N-fertility is not fully documented. The BNF is a sustainable and low-cost alternative to inorganic fertilizers in smallholder farming systems. Nonetheless, it is underutilized in soils of Lake Victoria basin partly due to insufficient information on the diverse populations of rhizobial strains and their N-fixation efficiency. To overcome these drawbacks, identifying resident and environmentally adaptable N-fixing rhizobia with superior symbiotic abilities and utilizing them in local cropping systems is necessary [11]. This is important because symbiotic efficiency of rhizobia is strongly influenced by edaphic and environmental factors [12][13].

II. MATERIALS AND METHODS

The study was conducted in glasshouse and screenhouse pot experiments at Kenya Forestry Research Institute's headquarters at Muguga, Nairobi. Soil samples used in pot experiments were collected from farmers' fields in Port Victoria in Busia County, Kisumu in Kisumu County, Kendu bay in Homabay County and Karungu in Migori County. A map of the sites is shown in Fig. 1 and site characteristics are shown on Table 1.

Glass house inoculation experiments at KEFRI headquarters

Authentication and symbiotic efficiency glasshouse pot experiments were arranged in Completely Randomized Design (CRD) with the two soybean varieties or the two bambara groundnut landraces as treatments in four replicates. Sixty four and 42 rhizobial colonies isolated from bambara groundnuts and soybeans respectively were evaluated by 16S rRNA gene sequences and grouped according to the maximum identity of the genus with those at the NCBI gene bank.

Table 1. Characteristics of soil sampling sites and soil types

Site	Site location	Agro-climatic zones	Soil type
Kisumu	0^0 6′0N $34^0$45′ 0E	Sub-humid lower midland	Clay loam
Karungu	$0^0$51′ 0S 34^0 8′ 60E	Semi-humid lower midland	Clay loam
Kendu bay	$0^0$22′ 0S $34^0$38′ 60E	Semi-humid lower midland	Sandy loam
P. Victoria	0^0 6′ 0N 33^0 58′ 0E	Sub-humid lower midland	Sandy loam

Source: Jaetzold *et al.*[14]

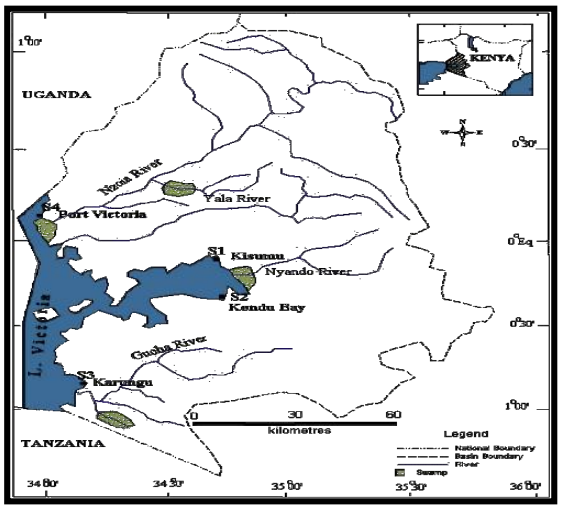

Fig.1. A map showing four sites within Lake Victoria basin where soil samples used to trap rhizobia were obtained.

Based on the results, 18 bambara isolates and 16 soybean isolates representative of all the groups identified were selected for determination of symbiotic status. One strain (*Bradyrhizobium spp*. KFR259) from Dr. David Odee's rhizobial collection at Kenya Forestry Research Institute's headquarters in Muguga and a commercial strain *Bradyrhizobium japonicum* USDA110 were chosen as reference strains based on previous reports that bambara groundnuts and soybeans nodulates better with *Bradyrhizobium spp*.[15].

Seeds were prepared as previously described and spread on water-agar (4:1) plates. The plates were inverted and incubated in dark cabinets at 25ºC for germination and were considered to be successfully germinated when their radicles reached the same length as the seed or longer. Improvised Leonard jars were prepared by sterilizing in 5% sodium hypochlorite solution for 15 minutes. Vermiculite obtained from a commercial supplier in Nairobi's industrial area was sterilized at 121ºC for 1 hr and the pH normalized to near neutral using 5% $CaCO_3$ solution and then filled in the jars. Each filled jar bottom was covered with absorbent sterile

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cotton wool to allow free flow of solutions while limiting contamination.

Two seedlings of each variety/landrace were aseptically transferred into each vermiculite pot at a depth of 5 mm using sterile pipette tips. The inoculum was prepared by streaking each isolate on freshly prepared YEMA plates. 1 ml of the pure culture was aseptically picked, re-suspended into sterile bottles containing 10 ml of Yeast Extract Mannitol broth and left on a shaker for 2 days. Eight replicates per isolate were inoculated directly around seedling hypocotyls 3 days after establishment using sterile disposable pipette tips. On each occasion, 1 ml (about 10^8 cells) of bacterial suspension was used to inoculate each seedling. Immediately after inoculation, vermiculite surfaces were covered with steam sterilized sand to inhibit contamination.

Growing conditions, data collection and harvesting

The experiments to evaluate symbiotic status of selected isolates were conducted in glasshouse-controlled conditions at 16/8 hr light/dark cycle, 25/18 °C day/night temperature and 70% relative humidity. The test seedlings and the negative controls (-N) were watered twice each week using 20 ml of sterile N-free nutrient solution [16] but the solution was increased to 40 ml after 2 weeks of growth. The positive controls (+N) were supplied with 10 ml of 0.5 M KNO_3 solution each week to supply the plants with nitrogen. After 45 days, the growth media was gently shaken off the plants, the roots carefully washed and the number of well-formed nodules counted. Nodules (when available), roots and shoots from the same pot were considered to belong to the same unit and were put in separately marked 2×1.75 cm zip-lock polythene bags for laboratory analysis. Fresh weights in grams per plant was taken in four replicates and recorded on an analytical electronic balance (The Lab Depot Inc, USA). Nodules, shoots and roots were enclosed in aluminium foil and inserted in an oven set at 70ºC for 1-2 days and dry weights obtained on an analytical electronic balance (The Lab Depot Inc, USA).

Representative shoot samples were ground using a mortar and pestle and submitted to Kenya Industrial Research and Development laboratories in Nairobi for analysis of N-content using the Kjeldahl method. The procedure used was as follows; Approximately 1 g of the sample was placed in a digestion flask and 1.5 g of potassium sulfate, 0.04 g anhydrous copper sulfate and 1.0 g alundum granules was added followed by 2.0 ml of sulfuric acid. The flask was placed on preheated burner and heated for 90 min until white fumes appeared while swirling gently. The mixture was cooled to room temperature while adding 250 ml of distilled water. Three drops of tributyl citrate was added then 45% sodium hydroxide solution drop by drop. The flask was immediately connected to a distillation apparatus and distilled at 25ºC until at least 150 ml distillate was collected in titrating flask and N concentration determined.

III. RESULTS

Glasshouse Authentication Experiments of Rhizobial isolates

The population of rhizobia from the soils based on the MPN results were; 1.7×10^3, 2.0×10^3, 1.9×10^3 and 1.5×10^3 for Port Victoria, Kisumu, Kendu bay and Karungu respectively. Host-strain interaction test results are shown in table 2. Out of the 64 bambara groundnut isolates, only 24 formed nodules with landrace KAKC out of which 7 were highly effective, 9 were partly effective while 8 were ineffective. Eighteen isolates were able to form nodules on landrace BUSB, but only 6 highly effective nodules were observed. In particular, soybean Variety 'Safari' was quite selective in nodulation potential failing to form nodules with 36 out of the 42 isolates. Only 2 isolates formed highly effective nodules on 'Safari' while effective nodules were observed in plants inoculated with 4 isolates. A distinction was observed on Variety SB19 which produced nodules with 22 isolates in which 9 were effective and 4 formed highly effective nodules. Majority of effective isolates on bambara groundnuts and soybeans were derived from Kisumu and Karungu soils.

Symbiotic Status of Selected Rhizobial Isolates in Glasshouse Conditions

1. **Performance of Selected Isolates on Bambara Groundnuts**

Glasshouse inoculation experiments on two bambara groundnut landraces with 18 isolates from this study and two reference strains (*Bradyrhizobium spp*. strains KFR259 and USDA110) resulted in variation in nodule, shoot, root and total plant biomass factors evaluated as shown in Table 3. There was a highly significant ($p<0.05$) effect of isolate inoculation on *number of nodules per plant*, although no clear landrace difference ($p>0.05$) was observed on this factor. Plants inoculated with isolates BAMKis1, BAMKis8, BAMKis4 and BAMKar3 which were genetically identified as *Bradyrhizobium spp*. produced highly effective and significantly ($p<0.05$) the highest mean *nodule number per plant* (above 20) followed by two isolates BAMKbay8 and BAMsp3 identified as *Burkholderia spp*. also giving above 20 nodules per plant.

Results of mean *nodule number per plant* obtained from the six isolates compared favorably with those obtained from the reference strains *Bradyrhizobium spp*. strain KFR259 and *Bradyrhizobium japonicum* strain USDA110, which produced an average of above 30 nodules per plant on landrace KAKC with the highest value of 49 nodules occurring on plants inoculated with strain USDA110. One isolate from Kisumu soils BAMKis1 genetically identified

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as *Rhizobium spp*. produced highly effective nodules on both landraces which numbered above 30, outperforming some of the *Bradyrhizobia spp*. (BAMKis8 and BAMKar3) that produced below 25 nodules per plant. Nodules classified as effective based on internal nodule colour were mostly found in plants inoculated with isolates genetically identified as *Rhizobium* (BAMKar2, BAMKar1 and BAMKis2 – each giving below 20 nodules per plant).Inoculation with isolates identified as *Agrobacterium spp*. resulted in no nodules (BAMKar9, BAMsp5 and BAMKbay9) except one isolate BAMKbay1 which produced above 10 nodules per plant most of which were effective.

Results on mean *nodule fresh* and *dry weights* followed a similar trend and posted highly significant response due to isolate inoculation ($p<0.05$), but there was no significant landrace effect on the two variables ($p>0.05$). The greatest mean *nodule fresh weight* of 0.477 g per plant was obtained from plants inoculated with isolate BAMKis1, producing slightly higher values ($p<0.05$) than both the reference strains, followed by plants inoculated with isolates BAMKis4 and BAMKis12 giving 0.449 g. Nodules retrieved from plants inoculated with isolate BAMKis1 had the highest mean *dry weight* of 0.073 g per plant followed by plants inoculated with BAMKis4 and BAMKis12 which ranged between 0.064 g and 0.065 g, values which were nearly identical to the mean *dry weight* of 0.067 g obtained from landrace KAKC plants inoculated with the reference strain USDA110 ($p<0.05$).

The mean *root* and *shoot dry weights* did not give a significant ($p>0.05$) difference due to inoculation but numerically the highest root dry weight of 0.302 g per plant was surprisingly obtained from non-nodulated plants of landrace KAKC inoculated with BAMKar9 – (identified as *Agrobacterium* sp.), which was much higher than the results obtained from the +N un-inoculated positive control (averagely 0.25 g per plant for both landraces). Although not significantly different ($p>0.05$) numerically the highest shoot dry weights of 2.32 g and 1.59 g per plant were obtained from the +N control plants with all the plant appearing tall and exhibiting large leaves. The total plant biomass also showed no significant difference ($p>0.05$) due to inoculation having been obtained from both the root and shoot dry weights.

2. Performance of Selected Isolates on Soybeans

Glasshouse inoculation experiments of two soybean varieties (SB19 and 'Safari') with 16 isolates and two reference strains resulted in variation in plant growth as shown in Table 4. There was a significant ($p<0.05$) effect of isolate inoculation on the mean *number of nodules* obtained from both soybean varieties although there was no significant ($p>0.05$) inter-varietal difference for the same variable. Plants inoculated with isolates SoyKis3 and SoyKar2 identified as *Bradyrhizobium elkanii* had significantly ($p<0.05$) the highest nodule number per plant, giving over 20 highly effective nodules from SB19 and 'Safari'. Inoculation of SB19 with isolates SoyKar2 and SoyKar4 (*Bradyrhizobium spp*.) from Karungu soils resulted in effective nodules which ranged from 13.8 to 16.5 nodules per plant, significantly higher ($p<0.05$) than plants inoculated with isolates from Kendu bay soils which had less than 10 nodules per plant. It was evident that most isolates from Port Victoria soils (Soysp2, Soysp3 and Soysp4 – within *Rhizobium* and *Agrobacterium* genus) were incompatible with both soybean varieties used in this study as the isolates did not form any nodules; with only Soysp1 producing six nodules on SB19.

A comparison of mean *nodule fresh weight* revealed no significant ($p>0.05$) varietal differences between non-inoculated SB19 and 'Safari', but there was a significant response to inoculation in both varieties ($p<0.05$). The greatest mean nodule fresh weight of 0.350 g was obtained from varieties SB19 and 'Safari' inoculated with SoyKar1 while the least nodule fresh weight was obtained from plants inoculated with SoyKbay2 and SoyKbay1 both giving a similar value of 0.005 g for SB19. Plants inoculated with SoyKis1 and the reference strain KFR259 produced higher nodule dry weight ($p<0.05$) giving a similar value of 0.086 g.

A significant ($p<0.05$) variation in mean *shoot dry weight* occurred with +N positive control plants producing the highest value of 1.434 g on the specific 'Safari' variety, followed by the same treatment on Variety SB19 with a value of 1.159 g($p<0.05$). Inoculation with the reference strain USDA110 and one isolate SoyKis1 resulted in above 1 g shoot dry weight, significantly higher than the values obtained from all the other isolates. No significant difference ($p>0.05$) due to treatment effects occurred between the varieties with regard to root dry weight. The mean *total plant biomass* was significantly ($p<0.05$) affected by inoculation with the Variety 'Safari' posting values of above 1.0 g with most of the isolates which produced nodules. However, +N plants produced the highest mean plant biomass of 1.704 g.

Table 2. Effectiveness tests of indigenous rhizobial isolates on two bambara groundnut landraces and two soybean varieties

Bambara groundnut isolates						Soybean isolates					
Isolate Identity	Landrace		Isolate Identity	Landrace		Isolate Identity	Variety		Isolate Identity	Variety	
	KAKC	BUSB		KAKC	BUSB		SB19	SAF		SB19	SAF
BAMKis1	HE	HE	BAMKbay12	I	I	SoyKis1	HE	E			
BAMKis2	E	PE	BAMKbay13	X	X	SoyKis2	HE	E	Soysp1	I	X
BAMKis3	PE	PE	BAMKbay14	X	X	SoyKis3	HE	HE	Soysp2	X	X
BAMKis4	HE	PE				SoyKis4	E	E	Soysp3	X	X
BAMKis5	X	X	BAMsp1	E	E	SoyKis5	I	X	Soysp4	X	X
BAMKis6	PE	PE	BAMsp2	E	E	SoyKis6	X	X			
BAMKis7	X	X	BAMsp3	HE	HE	SoyKis7	X	X	SoyKar1	E	E
BAMKis8	HE	H	BAMsp4	I	I	SoyKis8	I	X	SoyKar2	HE	HE
BAMKis9	X	X	BAMsp5	X	X	SoyKis9	X	X	SoyKar3	E	E
BAMKis10	PE	X	BAMsp6	X	X	SoyKis10	X	X	SoyKar4	E	E
BAMKis11	X	X	BAMsp7	E	X	SoyKis11	X	X	SoyKar5	X	X
BAMKis12	HE	HE	BAMsp8	I	X	SoyKis12	I	X	SoyKar6	X	X
BAMKis13	X	X	BAMsp9	I	X	SoyKis13	X	X	SoyKar7	X	X
BAMKis14	X	X	BAMsp10	X	X	SoyKis14	X	X	SoyKar8	I	X
BAMKis15	X	X	BAMsp11	X	X	SoyKis15	X	X	SoyKar9	X	X
BAMKis16	X	X	BAMsp12	PE	PE	SoyKis16	X	X	SoyKar10	X	X
BAMKis17	X	X				SoyKis17	X	X	SoyKar11	X	X
BAMKis18	I	X	BAMKar1	E	E	SoyKis18	X	X	SoyKar12	X	X

Bambara Groundnut Isolates						Soybean isolates					
Isolate	Landrace		Isolate	Landrace		Isolate	Variety			Variety	
Identity	KAKC	BUSB	Identity	KAKC	BUSB	Identity	SB19	SAF	Isolate Identity	SB19	SAF
BAMKis19	X	X	BAMKar2	E	E	SoyKis19	I	X			
BAMKis20	X	I	BAMKar3	HE	HE	SoyKis20	X	X			
BAMKis21	X	X	BAMKar4	X	X	SoyKis21	X	X			
BAMKis22	X	I	BAMKar5	X	I	SoyKis22	X	X			
			BAMKar6	I	X						
BAMKbay1	X	X	BAMKar7	X	X	SoyKbay1	I	X			
BAMKbay2	X	X	BAMKar8	PE	E	SoyKbay2	E	X			
BAMKbay3	X	X	BAMKar9	X	X	SoyKbay3	I	X			
BAMKbay4	PE	PE	BAMKar10	X	X	SoyKbay4	I	X			
BAMKbay5	X	I	BAMKar11	X	X						
BAMKbay6	I	I	BAMKar12	X	X						
BAMKbay7	X	I	BAMKar13	X	X						
BAMKbay8	HE	HE	BAMKar14	X	X						
BAMKbay9	X	X	BAMKar15	PE	PE						
BAMKbay10	X	X	BAMKar16	X	X						
BAMKbay11	PE	PE									

NOTE: Isolates from the same site have the same colour regime.

Sites of origin;
Kis– Kisumu isolates
Kbay – Kendu bay isolates
Sp – Port Victoria isolates
Kar– Karungu isolates

Host strain interactions
HE - Highly effective (>20 nodules /plant)
E– Effective (>10 and < 20 nodules /plant)
PE - Partly effective (<10 and >5 nodules /plant)
I– Ineffective (< 5 nodules /plant)
X - No nodulation (0 nodules /plant)

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Table 3: Symbiotic status of 18 test rhizobial isolates, two reference strains and +N and –N controls on two bambara groundnut landraces (KAKC and BUSB) in glasshouse pot experiments

	Treatment /Isolate inoculant	NNO		NFW (g)		NDW (g)		RDW (g)		SDW (g)		TBM (g)	
		Landrace		Landrace		Landrace		Landrace		Landrace		Landrace	
		KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB
	+ N	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.200	0.263	2.121	1.591	2.321	1.854
	- N	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.142	0.154	0.328	0.390	0.470	0.544
	USDA110	49.00^{a}	32.00^{a}	0.387^{ab}	0.421^{ab}	0.064^{a}	0.067	0.168	0.288	1.005	1.143	1.236	1.498
	KFR 259	34.75^{b}	20.25^{b}	0.286^{b}	0.320^{bc}	0.044^{b}	0.044	0.186	0.239	0.661	0.889	0.891	1.171
	BAMKis12	30.50^{b}	26.75^{a}	0.203^{b}	0.444^{ab}	0.035^{bc}	0.065	0.149	0.292	0.896	1.152	1.081	1.510
	BAMKis8	22.71^{c}	20.50^{b}	0.319^{ab}	0.340^{ab}	0.041^{b}	0.049	0.172	0.289	1.062	1.031	1.275	1.370
	BAMKis4	37.23^{b}	27.75^{a}	0.306^{ab}	0.449^{ab}	0.053^{ab}	0.064	0.186	0.286	0.795	1.055	1.033	1.405
	BAMKbay8	21.25^{c}	22.31^{a}	0.314^{ab}	0.301^{bc}	0.044^{b}	0.047	0.159	0.198	0.842	0.791	1.145	1.036
	BAMsp3	22.02^{c}	15.75^{bc}	0.173^{b}	0.214^{c}	0.026^{bc}	0.031	0.208	0.212	0.759	0.695	0.993	0.938
	BAMKis1	35.77^{b}	31.75^{a}	0.293^{ab}	0.471^{a}	0.040^{b}	0.073	0.149	0.265	0.896	1.242	1.085	1.581
	BAMKar3	25.79^{c}	25.50^{a}	0.428^{a}	0.350^{ab}	0.059^{ab}	0.064	0.199	0.215	1.296	0.734	1.755	1.013
	BAMKar2	15.50^{cd}	22.01^{b}	0.288^{b}	0.173^{d}	0.038^{b}	0.026	0.110	0.208	0.767	0.759	1.016	0.993
	BAMKar1	17.50^{cd}	8.25^{c}	0.258^{b}	0.020^{e}	0.094^{a}	0.003	0.186	0.167	0.735	0.809	1.015	0.981
	BAMKis2	10.49^{d}	18.50^{b}	0.219^{b}	0.277^{cd}	0.032^{bc}	0.027	0.107	0.236	0.954	0.692	1.392	1.124
	BAMKbay1	11.25^{d}	17.50^{bc}	0.211^{b}	0.184^{cd}	0.039^{b}	0.022	0.185	0.225	0.581	0.914	0.766	1.139
	BAMsp2	1.51^{e}	4.51^{d}	0.016^{c}	0.017^{e}	0.021^{bc}	0.004	0.241	0.188	0.661	0.937	0.932	1.129
	BAMsp1	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.258	0.233	0.519	0.643	0.777	0.877
	BAMKbay2	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.231	0.184	0.838	0.678	1.369	0.863
	BAMKar9	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.302	0.210	0.663	0.669	0.965	0.879
	BAMKbay9	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.279	0.184	0.701	0.678	0.980	0.863
	BAMKbay3	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.198	0.192	0.791	0.621	0.989	0.813
	BAMsp5	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.179	0.264	0.609	0.779	0.789	1.043
	SED	**0.013**		**0.095**		**0.025**		**0.069**		**0.067**		**0.178**	
	Var.	**2.95^{ns}**		**0.042^{ns}**		**0.011^{ns}**		**0.041^{ns}**		**0.105^{ns}**		**0.133^{ns}**	
LSD (0.05)	**Isol.**	**9.78***		**0.139***		**0.036***		**0.138^{ns}**		**0.348^{ns}**		**0.441^{ns}**	
	CV (%)	**18.0**		**30.8**		**15.6**		**16.9**		**16.4**		**16.1**	

NOTE*: Means followed by the same letter in a column are not significantly different* (* - Significant at p≤0.05)

+N – Nitrogen positive control; **-N** Un-inoculated negative control; **NNO** – Nodule number per plant; **NFW** – Nodule fresh weight; **NDW** – Nodule dry weight; **SDW** – shoot dry weight; **RDW** – Root dry weight; **TBM** – Total biomass; ***ns*** – not significant; **Var** – Variety; **Isol**. – Isolate.

Table 4. Symbiotic status of 16 test rhizobial isolates, two reference strains and +N and –N controls on two soybean varieties (SB19 and Safari) in glasshouse pot experiments

Treatment	NNO		NFW (g)		NDW (g)		SDW (g)		RDW (g)		TBM (g)	
	Variety		Variety		Variety		Variety		Variety		Variety	
	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF
+N	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	1.159^{a}	1.434^{a}	0.305	0.271	1.463^{a}	1.704^{a}
- N	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.281^{e}	0.216^{e}	0.258	0.189	0.439^{e}	0.405^{e}
USDA 110	28.5^{a}	30.0^{a}	0.399^{a}	0.314^{a}	0.083^{a}	0.100^{a}	0.855^{b}	1.042^{b}	0.203	0.207	1.142^{b}	1.349^{b}
KFR259	13.5^{c}	17.0^{b}	0.286^{bc}	0.268^{b}	0.086^{a}	0.058^{bc}	1.007^{a}	0.664^{d}	0.218	0.223	1.311^{a}	0.945^{cd}
SoyKis3	25.5^{a}	20.5^{b}	0.322^{b}	0.296^{ab}	0.066^{abc}	0.077^{a}	0.790^{bc}	0.890^{bc}	0.185	0.161	1.040^{bc}	1.128^{b}
SoyKar2	25.3^{a}	20.0^{b}	0.310^{b}	0.169^{c}	0.076^{ab}	0.043^{c}	0.752^{bc}	0.618^{d}	0.169	0.154	0.997^{bc}	0.815^{d}
SoyKis4	19.3^{b}	8.5^{d}	0.296^{bc}	0.131^{c}	0.056^{bc}	0.031^{c}	0.675^{c}	0.808^{c}	0.142	0.228	0.874^{cd}	1.067^{c}
SoyKis2	18.0^{bc}	12.3^{c}	0.339^{a}	0.229^{b}	0.078^{ab}	0.058^{bc}	0.729^{bc}	0.880^{bc}	0.174	0.155	0.981^{bc}	1.093^{bc}
SoyKar1	15.5^{bc}	15.5^{bc}	0.350^{a}	0.350^{a}	0.075^{ab}	0.085^{a}	0.757^{bc}	0.997^{b}	0.177	0.164	1.008^{bc}	1.245^{b}
SoyKis1	13.0^{c}	11.0^{cd}	0.233^{c}	0.287^{ab}	0.051^{bc}	0.086^{a}	0.576^{cd}	1.007^{b}	0.217	0.218	0.845^{cd}	1.311^{b}
SoyKar3	16.5^{bc}	0.0^{e}	0.243^{c}	0.000^{d}	0.016^{d}	0.000^{d}	0.657^{cd}	0.790^{c}	0.215	0.185	0.988^{bc}	0.975^{cd}
SoyKar4	13.8^{c}	0.0^{e}	0.208^{c}	0.000^{d}	0.047^{c}	0.000^{d}	0.557^{cd}	0.808^{c}	0.194	0.228	0.799^{cd}	1.036^{c}
SoyKbay1	0.5^{e}	0.0^{e}	0.005^{e}	0.000^{d}	0.001^{d}	0.000^{d}	0.545^{cd}	0.602^{d}	0.181	0.158	0.927^{c}	0.760^{d}
SoyKbay2	2.5^{e}	0.0^{e}	0.031^{e}	0.000^{d}	0.005^{d}	0.000^{d}	0.575^{cd}	0.763^{c}	0.301	0.162	0.881^{cd}	0.925^{c}
SoyKbay4	1.3^{e}	0.0^{e}	0.005^{e}	0.000^{d}	0.002^{d}	0.000^{d}	0.489d	0.635^{d}	0.177	0.199	0.666^{cde}	0.834^{cd}
Soysp1	6.3^{d}	0.0^{e}	0.141^{d}	0.000^{d}	0.027^{cd}	0.000^{d}	0.563^{cd}	0.689^{cd}	0.198	0.175	0.788^{cd}	0.864^{cd}
SoyKbay3	1.5^{e}	0.0^{e}	0.010^{e}	0.000^{d}	0.002^{d}	0.000^{d}	0.600^{cd}	0.649^{d}	0.232	0.147	0.833^{cd}	0.796^{d}
Soysp2	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.521^{cd}	0.671^{d}	0.162	0.159	0.683^{cd}	0.830^{cd}
Soysp3	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.421^{de}	0.647^{d}	0.205	0.100	0.626^{de}	0.747^{d}
Soysp4	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.563^{cd}	0.772^{cd}	0.288	0.191	0.851^{cd}	0.963^{c}
SED	**0.737**		**0.044**		**0.004**		**0.113**		**0.048**		**0.032**	
LSD(0.05) Var	**1.458^{ns}**		**0.021^{ns}**		**0.007^{ns}**		**0.056****		**0.021^{ns}**		**0.064^{ns}**	
Isol.	**4.61***		**0.065***		**0.023***		**0.178***		**0.068^{ns}**		**0.266***	
CV (%)	**49.5**		**48. 1**		**66.6**		**23.1**		**34.2**		**20.2**	

NOTE: *Means followed by the same letter in a column are not significantly different* (* - Significant at p≤0.05)

KEY:+N – Nitrogen positive control; **-N** Un-inoculated negative control; **NNO** – Nodule number per plant; **NFW** – Nodule fresh weight; **NDW** – Nodule dry weight; **SDW** – shoot dry weight; RDW – Root dry weight; **TBM** – Total biomass; ***ns*** – not significant; **Var** – Variety; **Isol**. – Isolate.

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IV. DISCUSSION

Inoculation of Bambara Groundnuts with Selected Rhizobial Isolates in the Greenhouse

Glasshouse inoculation of bambara groundnut plants resulted in significant variations in some plant growth parameters particularly in increased nodule formation. All treatments inoculated with *Bradyrhizobium spp*. isolates produced highly effective nodules with intensely pink internal nodule colours, with some strains (BAMKis12 and BAMKis4) outperforming *Bradyrhizobium japonicum* strain USDA110 in accumulation of plant biomass values. These findings imply a higher rate of symbiotic efficiency and N-fixation of strains BAMKis12 and BAMKis4 than the commercial strains indicating their elitist status as prospective sources of inoculants for local use as bio fertilizers.

Another remarkable outcome of this study was the occurrence of two *Burkholderia* spp. strains (BAMKbay8 and BAMsp3) as N-fixing associates of bambara groundnuts which was confirmed by highly effective and above 20 nodules per plant in the glasshouse experiments. Isolate BAMKbay8 outperformed the commercial strain USDA110 and nearly equalled the reference strain KFR259 in symbiotic performance. The N-fixation ability of some members of the genus *Burkholderia* was first proposed by Moulin *et al*. [17], confirmed by Chen *et al*.[18] and later symbiotic strains were distinctly established [19].

From the findings of Vandamme *et al*. [20], the Cape Fynbos in South Africa was listed as a major diversity centre of the N-fixing *Burkholderia* strains. The 16S rRNA gene phylogeny of the two isolates (BAMKbay8 and BAMsp3) from this study depicted their close relations to *Burkholderia tuberum* strain DUS833 and *B. phymatum* strain JVNU IL24 with the former having been isolated from the root nodules of *Aspalathus callosa* in South Africa. According to Ngugi *et al*. [21] bambara groundnuts landraces cultivated in Kenya are of West and South African descent and were introduced into East African farming systems through Uganda and Tanzania during the migration of Bantus, or later on during inter-border trading between the East African countries. It is therefore plausible to argue that the N-fixing *Burkholderia* strains found in this study may have been introduced together with the bambara groundnut germplasm during this period. Furthermore, the two isolates occurred in the soils of Port Victoria and Kendu bay which were active inter-border trading points through Lake Victoria. The absence of *Burkholderia* spp. isolates from Kisumu and Karungu soils may be due to its preference to less acidic soils.

Results of inoculation with isolates identified as *Agrobacterium* spp. resulted in one isolate (BAMKbay1) which produced effective nodules. This was unexpected since the N-fixation ability of this genus is still disputed as this process is mediated by *nif* and *nod* genes which are supposedly absent in this group [22]. Thus, the effective nodules produced by BAMKbay1 may have been as a result of acquisition of *nif* genes through lateral transfer of the *Sym* plasmid from other groups which possess the *nif* genes naturally [23]. Although *Agrobacterium* is known to be an effective endophytic inducer of tumors in plant roots [24], it is possible that the tumor inducing *Ti* plasmid responsible for the pathogenic structures were lost after it gained the genes for N_2-fixation. Indeed, the isolate may have acquired both *nif* and *nod* genes (within the *Sym* plasmid) which enabled it to produce the high number of nodules recorded. On the other hand, inoculation with isolate BAMKar9 also genetically identified as *Agrobacterium spp*. produced enlarged root tissues which appeared swollen that resulted in the higher root dry weight values than those obtained from plants under +N control treatment. Possibly, isolate BAMKar9 may have retained the tumor inducing *Ti* plasmid which resulted in larger roots that may have contributed to the observed difference in root biomass.

Some of the most promising strains for inoculation of bambara groundnuts established in this study were; BAMKis12, BAMKis8, BAMKis4 and BAMKar3 (*Bradryrhizobium spp*.) and BAMKbay8 and BAMsp3 (*Burkholderia spp*) which produced highly effective nodules and high plant biomass values. These strains have potential use as bio-fertilizers in the production of bambara groundnuts although they were relatively not well distributed in the four soils used for isolation unlike isolates identified as *Agrobacterium* and *Rhizobium* which occurred in all the soils. Possibly, stiff competition for infection and nodulation amongst different strains may have favoured the fast-growing genera (*Rhizobium* and *Agrobacterium*) which are known to have better rates of competition for nodule occupancy [25]. Thus, bambara groundnut is arguably more compatible with the genus *Bradyrhizobium* and *Burkholderia* but are seemingly out-competed for nodule occupancy in the local soils by the less/ineffective *Rhizobium* and *Agrobacterium* respectively. Conversely, since competitive ability and nodule occupancy is influenced by the prevailing status of the media [26]. The pH of the vermiculite used as the growing media was normalized to near neutral, this may have impacted negatively on some of the acid tolerant strains.

Although there were varying responses in nodulation of bambara groundnut plants due to inoculation with different isolates and the reference strains, it was surprising to find no significant difference in factors such as shoot and root dry weights. This may be attributed to the slow rate of N-nutrition of some bambara groundnut cultivars that which have previously been shown to convert only 33% of symbiotic N into the plant organic material [27].

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Conversely, the period taken to harvest the test plants (45 days) for evaluation of dry weight may not have been sufficient for assimilation of fixed N that could result in significant difference in plant dry matter.

Inoculation of Soybeans with Selected Rhizobial Isolates

Results of glasshouse inoculation with 16 indigenous soybean rhizobia and the reference strains *Bradyrhizobium japonicum* USDA110 and *Bradyrhizobium spp*. KFR256 resulted in significant differences in all the growth attributes evaluated except in root dry weight. Variety 'Safari' was more selective forming nodules with only five of the isolates compared to SB19 which formed nodules with ten isolates. The best performing isolates were SoyKis3 and SoyKar2, genetically identified as *Bradyrhizobia* both comparing favorably with the reference strain *B. japonicum* strain USDA110, but outperforming *Bradyrhizobium spp.* strain KFR259. In terms of nodule formation, the two strains resulted in over 20 highly effective nodules for both SB19 and 'Safari', similar to the results obtained from strain USDA 110. According to Hirsch *et al*. [28], soybeans produce special isoflavonoids known as daidzein and genistein which are effective inducers of *B. japonicum nod* genes, but inhibit other rhizobial groups such as *S. meliloti nod* gene expression. This narrow host range was observed in this study as both SB19 and 'Safari' formed more nodules with indigenous *Bradyrhizobia* strains SoyKis3 and SoyKar2, as was previously observed by Broughton *et al*.[29].

Variety SB19 had a wider host range forming partially effective nodules with some non-*Bradyrhizobia* groups including SoyKis1 which was identified as *Rhizobium* sp. and SoyKar3 identified as *Mesorhizobium* spp. further confirming its promiscuous status. However, the symbiotic efficiency of the two strains (SoyKis1 and SoyKar3) was lower than that of strains identified as *Bradyrhizobia*. These findings indicate that SB19 may have a greater competition for nodule occupancy in cultivated soils with a wider range of rhizobial populations, which may negatively affect N-fixation. This challenge could be addressed by seed treatment of the promiscuous host with competent strains. This may provide a competitive advantage and result in higher nodulation [30].

V. CONCLUSIONS

Soybean Variety SB19 showed 'promiscuous' tendencies and formed effective nodules with rhizobia in the genera *Bradyrhizobium*, *Rhizobium* and *Agrobacterium* while 'Safari' was quite selective and formed very few nodules with isolates identified as *Bradyrhizobium*, which were mostly obtained from Kisumu soils. Both varieties SB19 and 'Safari' had better growth under glasshouse inoculation with isolates identified as *Bradyrhizobium spp*., although one *Rhizobium* isolate (SoyKis1) resulted in good nodulation of both varieties.

VI. RECOMMENDATIONS

Seed treatment of the two legumes with some isolates resulted in improved nodulation and better plant growth; in some instances, outperforming the commercial strain *Bradyrhizobium japonicum* USDA110. Isolates BAMKis12, BAMKis8, BAMKis4, BAMKbay8 and SoyKar2 are promising elite strains and are recommended for more host range tests as potential inoculants sources.

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